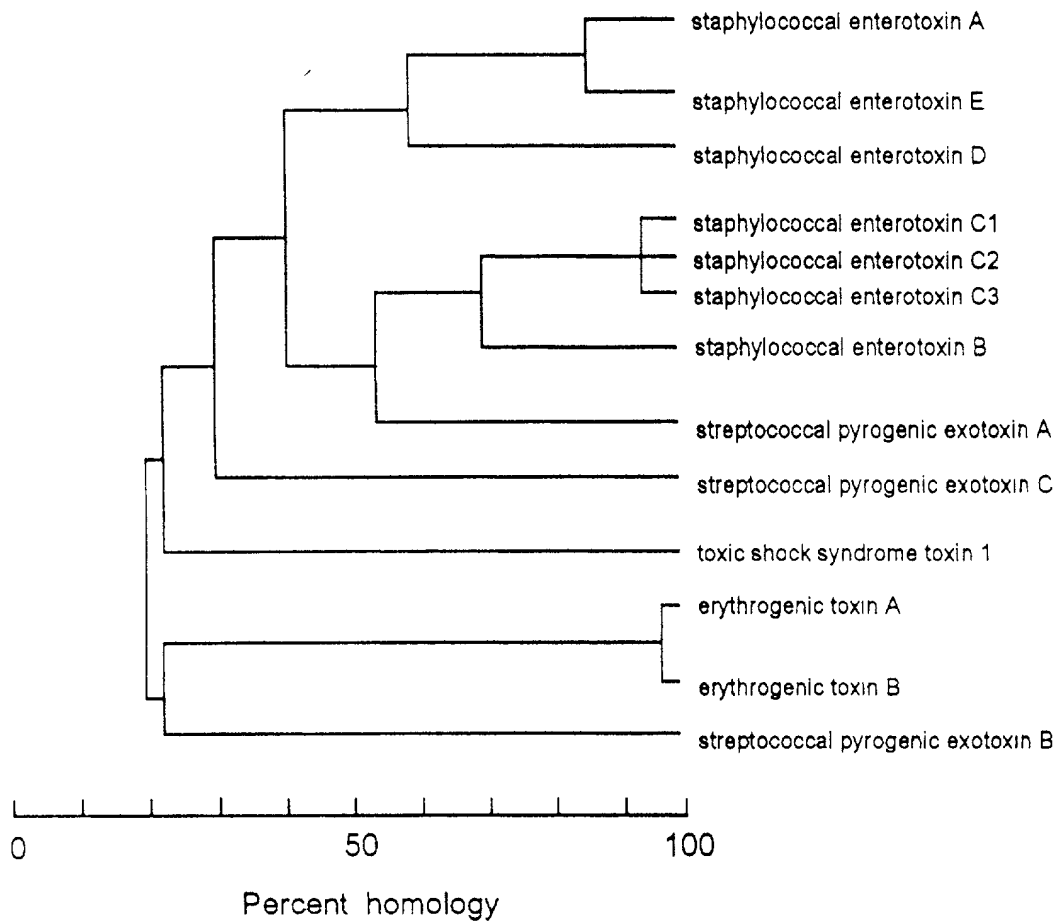


100  
90  
80  
70  
60  
50  
40  
30  
20  
10  
0



115

FIGURE 1

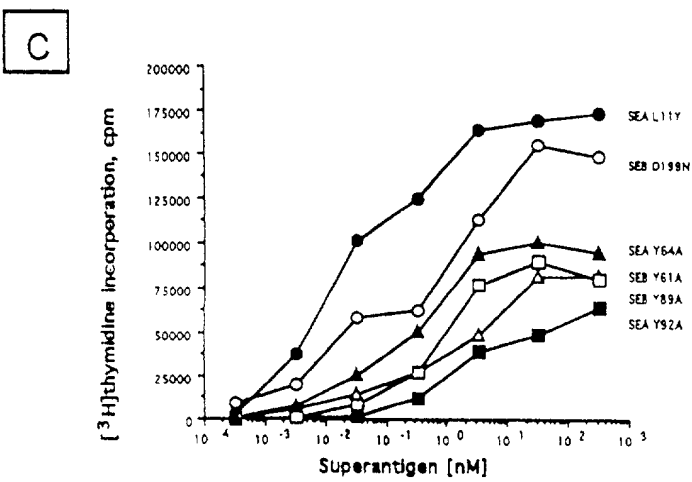
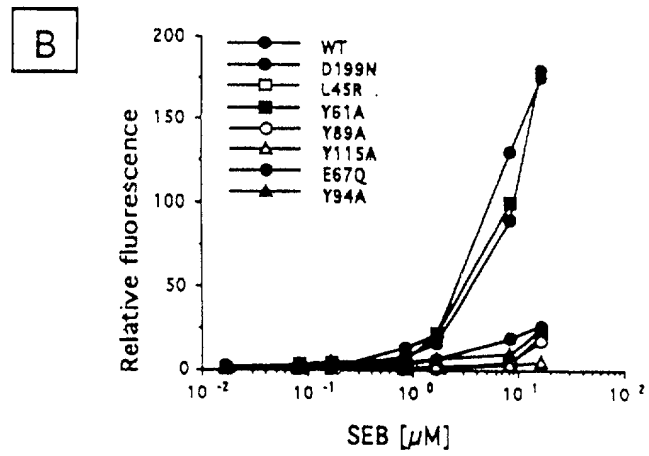
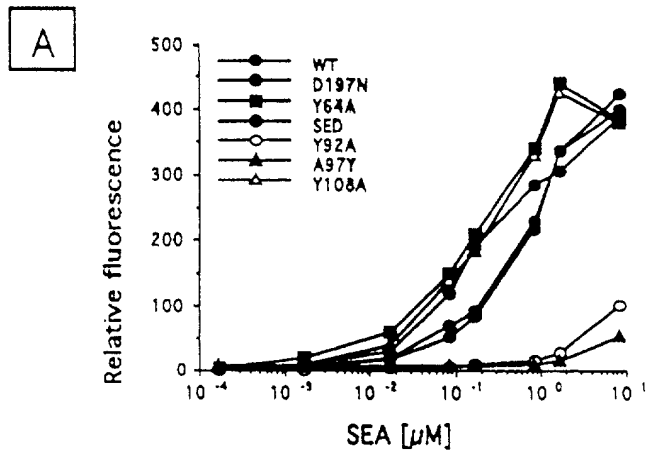


Fig. 2

	48	70	92	108																
SEA	...SHDQF	QHTILFKGFFTDH	SWYNDLLV	FDSKDI	VDKYK	GKKVDLYGAY	GYQCA	.....GGTPNK	TACM	GGVT	LHDN	NRL	TEKK							
SED	...TGQDF	ENTLLYK	FFTDL	INFEDLLI	FNSKEMA	QHFK	SKNVDV	PIR	SINCY	.....GGEIDR	TACT	GGVT	PHEG	NKL	KERKK					
SEE	...SDDQF	ENTLLFK	GFFTG	HPWYNDLLV	LGSKDAT	NKYK	GKKVDLYGAY	GYQCA	.....GGTPNK	TACM	GGVT	LHDN	NRL	TEKK						
SEB	...SIDQF	YFDLI	YSIK	DTKLG	NYDNVRV	RFKNKD	LADKYK	DKYVDV	FGAN	YYQCYF	SKKTND	INSHQT	DKRKT	CM	GGVT	EHGN	QOLD	.KY		
SEC1	...SVDKF	LAHDLI	YNI	SDKKL	KNYDKVK	TKT	LLNEGL	LAKKYK	DEVVDV	YGSN	YVNCYF	SSKDN	VGKV	TGG	..KT	CM	GGIT	KHEG	NHFD	NGNL
SEC2	...SVDKF	LAHDLI	YNI	SDKKL	KNYDKVK	TKT	LLNEDL	LAKKYK	DEVVDV	YGSN	YVNCYF	SSKDN	VGKV	TGG	..KT	CM	GGIT	KHEG	NHFD	NGNL
SEC3	...SVDKF	LAHDLI	YNI	SDKKL	KNYDKVK	TKT	LLNEDL	LAKKYK	DEVVDV	YGSN	YVNCYF	SSKDN	VGKV	TGG	..KT	CM	GGIT	KHEG	NHFD	NGNL
SPea	...SVDQL	SHDLI	YNV	SG...PNYD	KLKT	TKNQEM	ATLFK	DKNVDI	YGVE	YHLCYL	CENAE	.....RSACI	GGVT	NH	EGN	HLEI	PK			
TSST1	...VLDNS	GSMRIK	NTD	.....GSIS	LIF	FPSPY	YSPA	FTKGEK	VDLNTKR	KKSQHT	SEG	.....TYIHF	Q	SGVT	NT	EKL	PT	...P		

Fig. 3

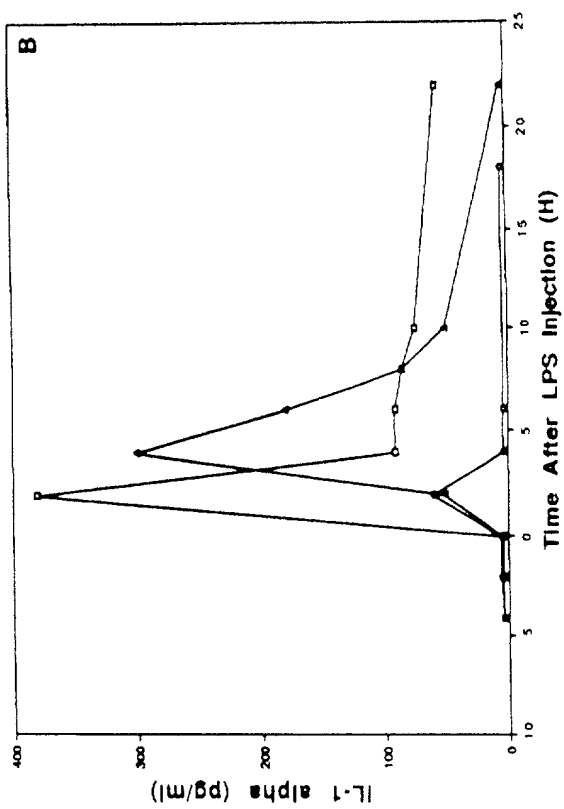
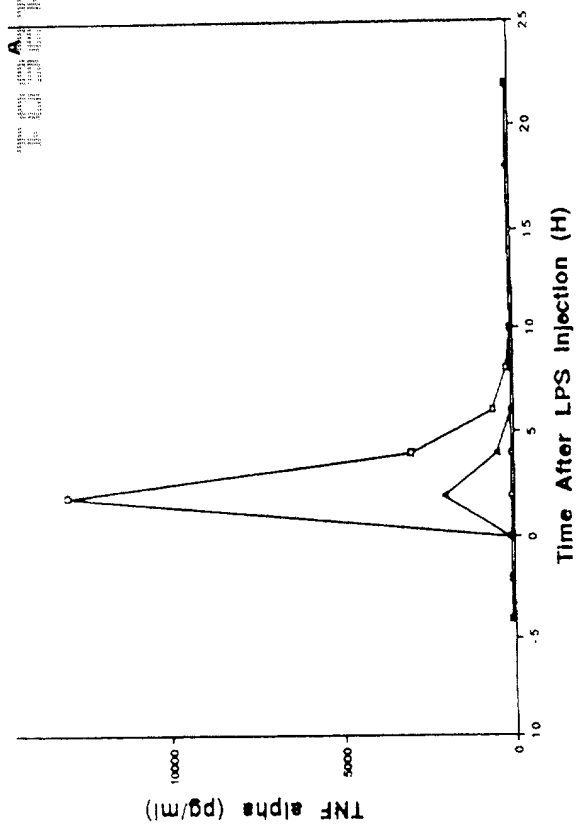
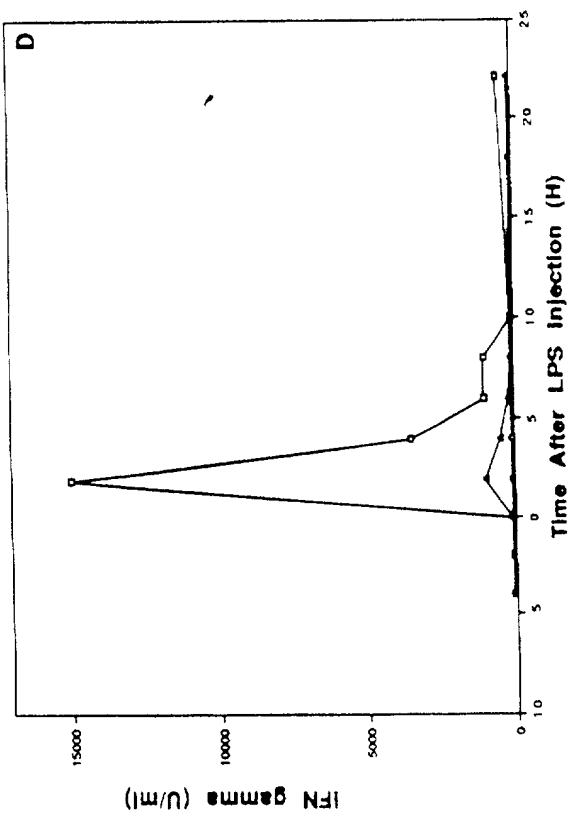
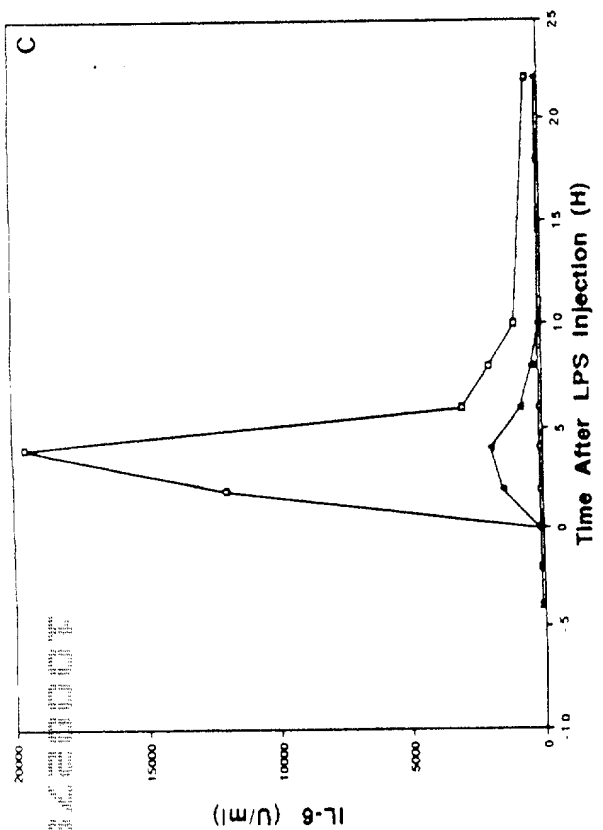


Fig 4

Figure 5: Bar graph showing [3H]-thymidine incorporation (CPM) for various conditions. The y-axis ranges from 0 to 150,000 CPM. The x-axis categories are WT-SEA, SEA K14E, SEA Y64A, SEA Y92A, Adjuvant, and Untreated. Error bars are shown for each bar.

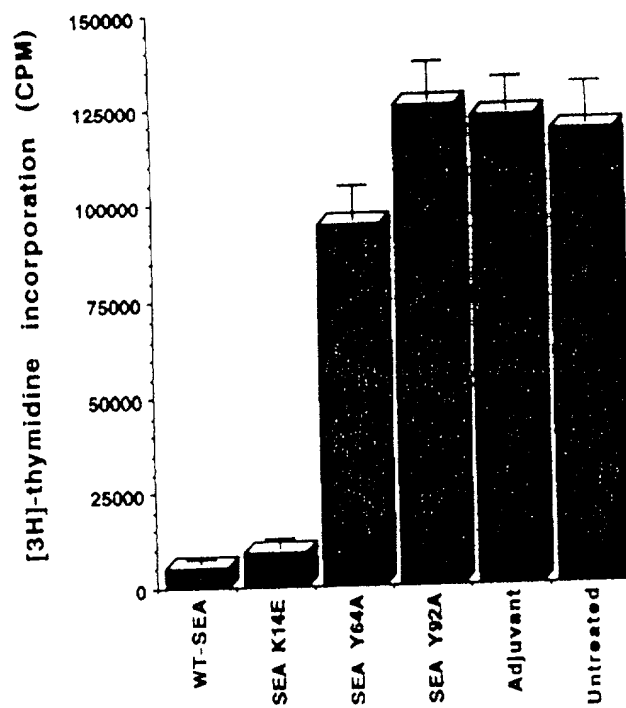


Fig. 5

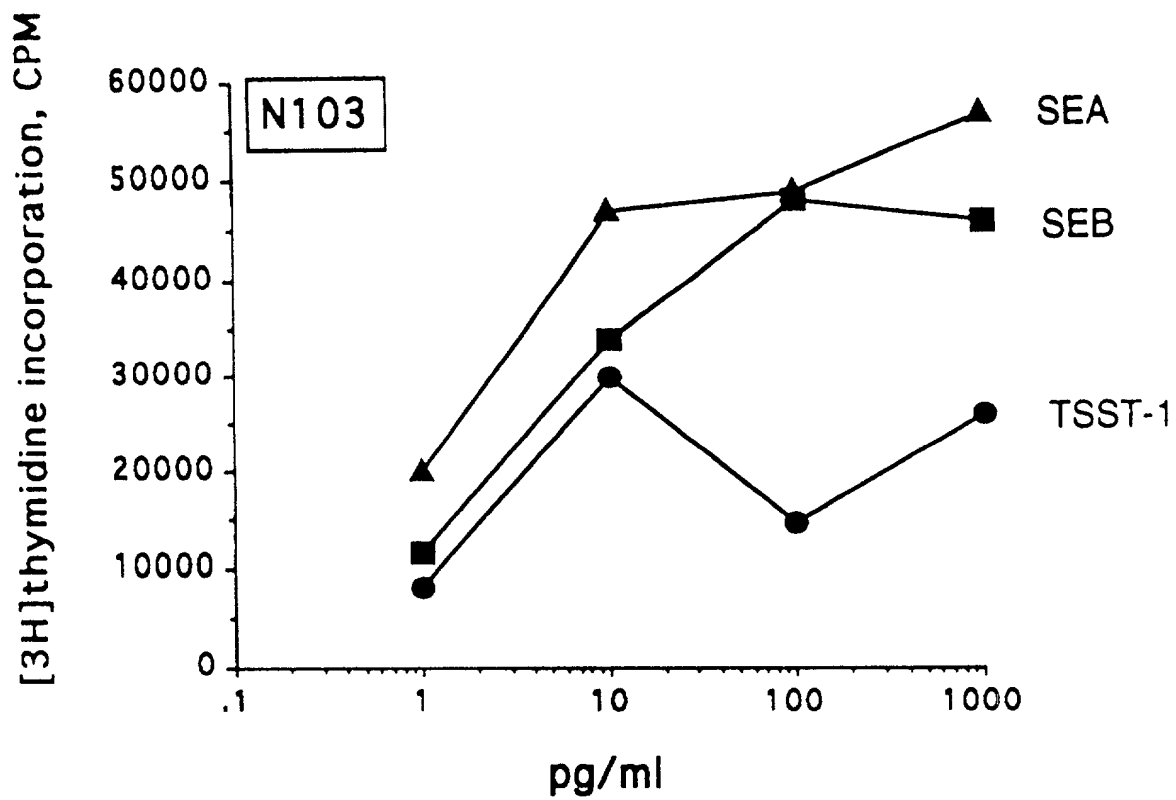
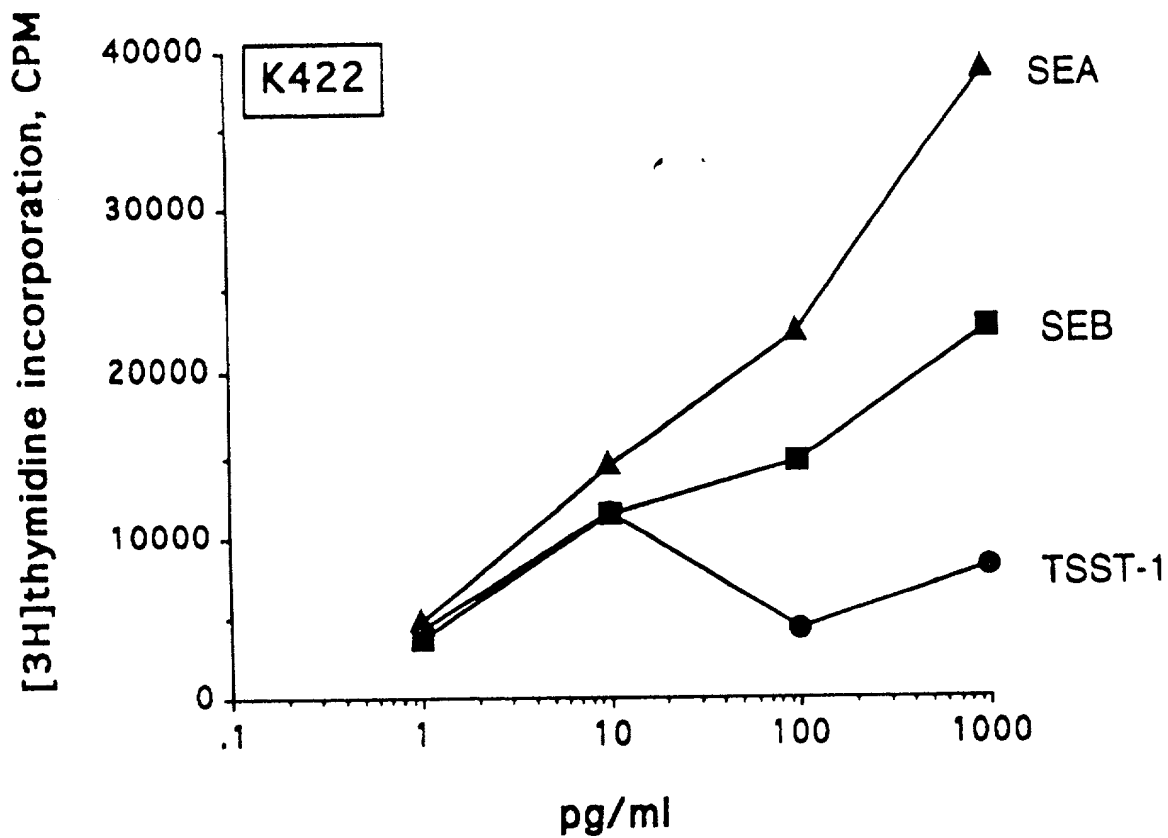
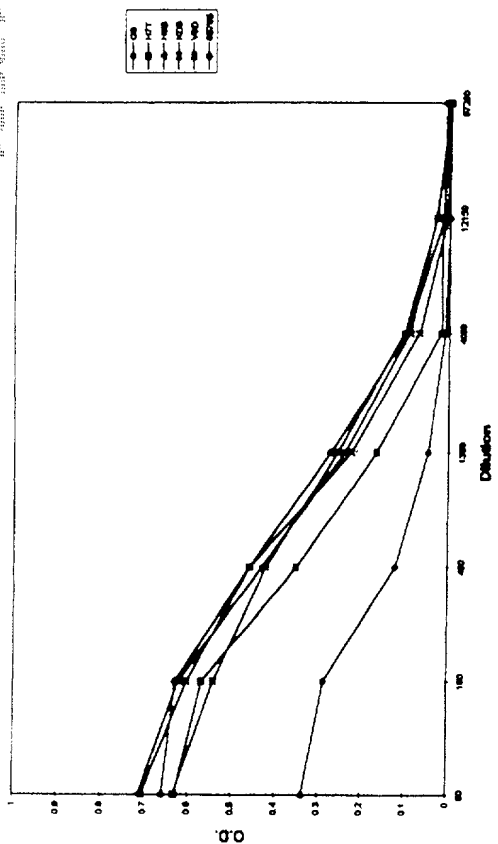
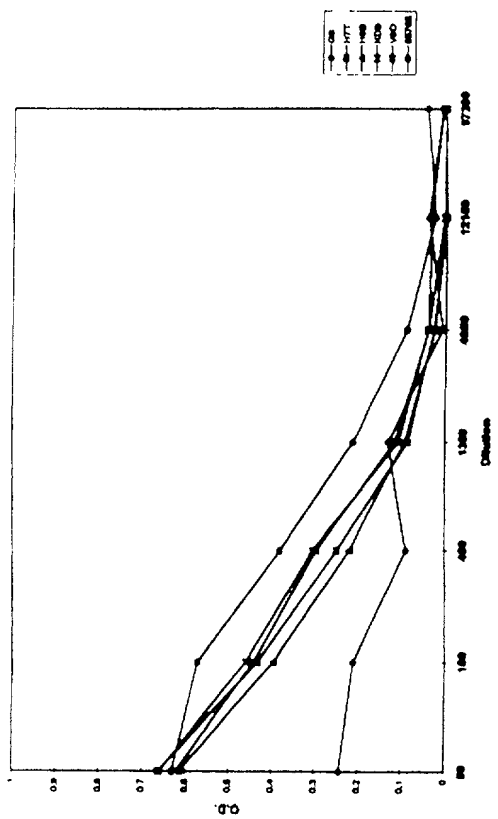


Fig. 6

Monkey Sera (3rd bleed) Response to SEA [0.2µg/well]



Monkey Sera (3rd bleed) Response to SEC [0.2µg/well]



Monkey Sera (3rd bleed) Response to SEC [0.2µg/well]

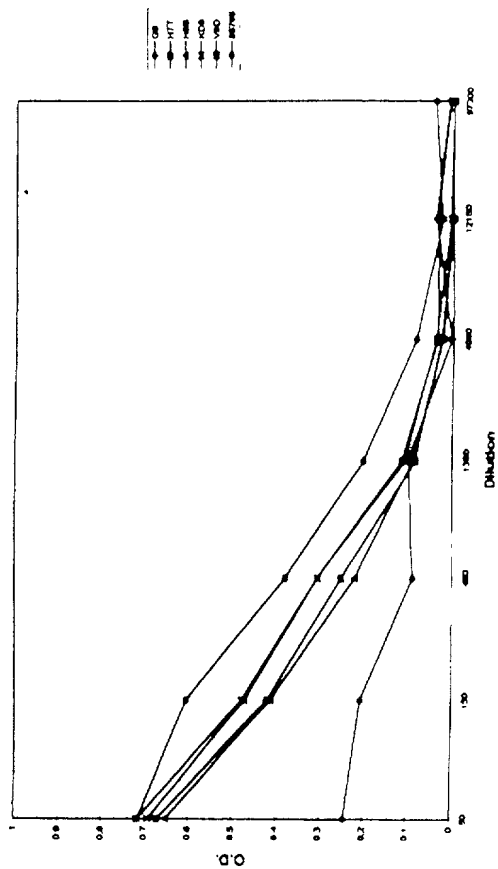
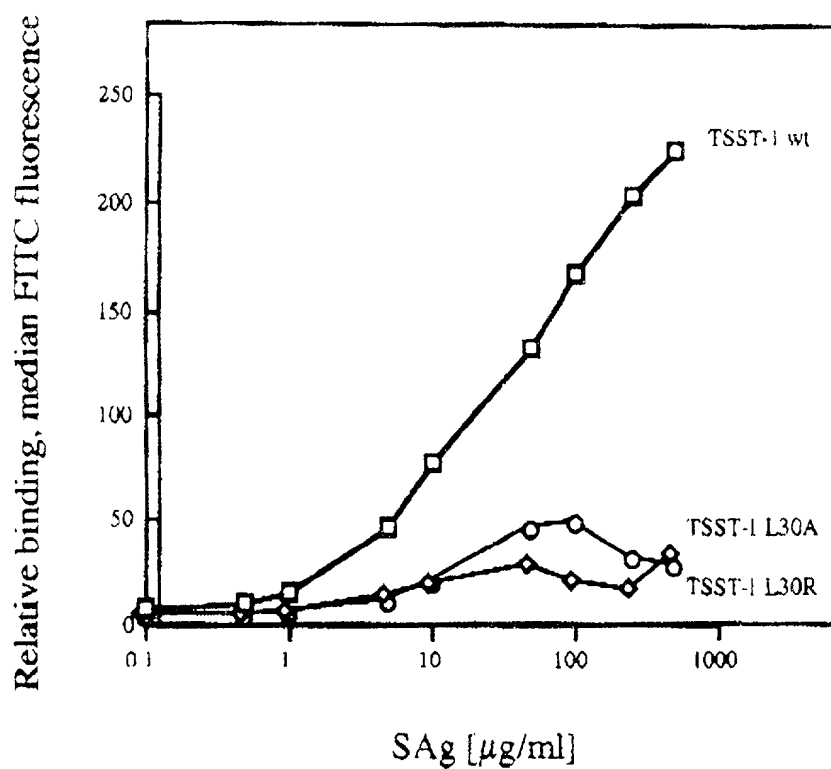


Fig. 7

A.



B.

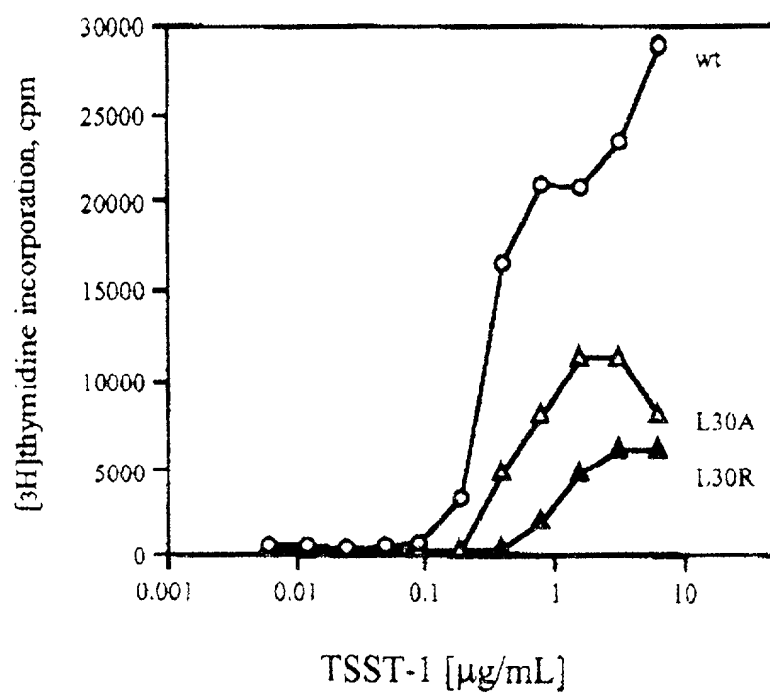
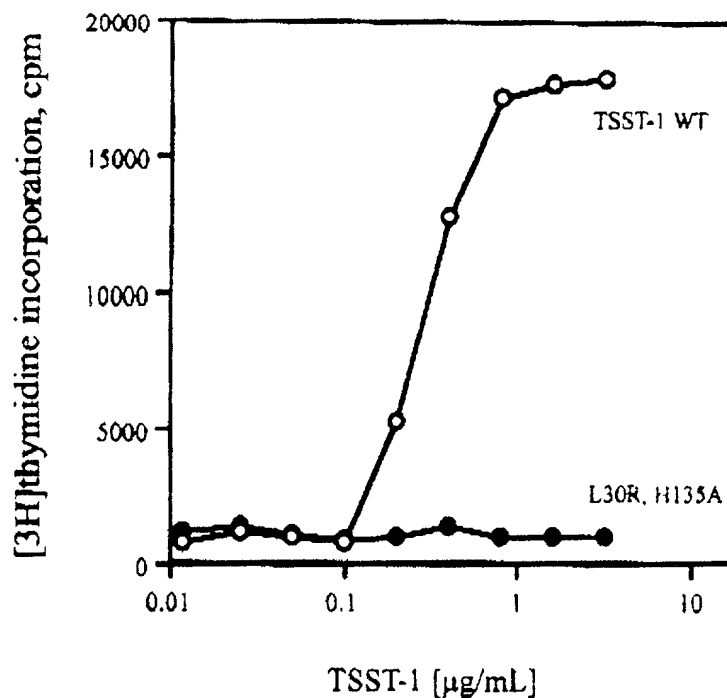


FIGURE 8

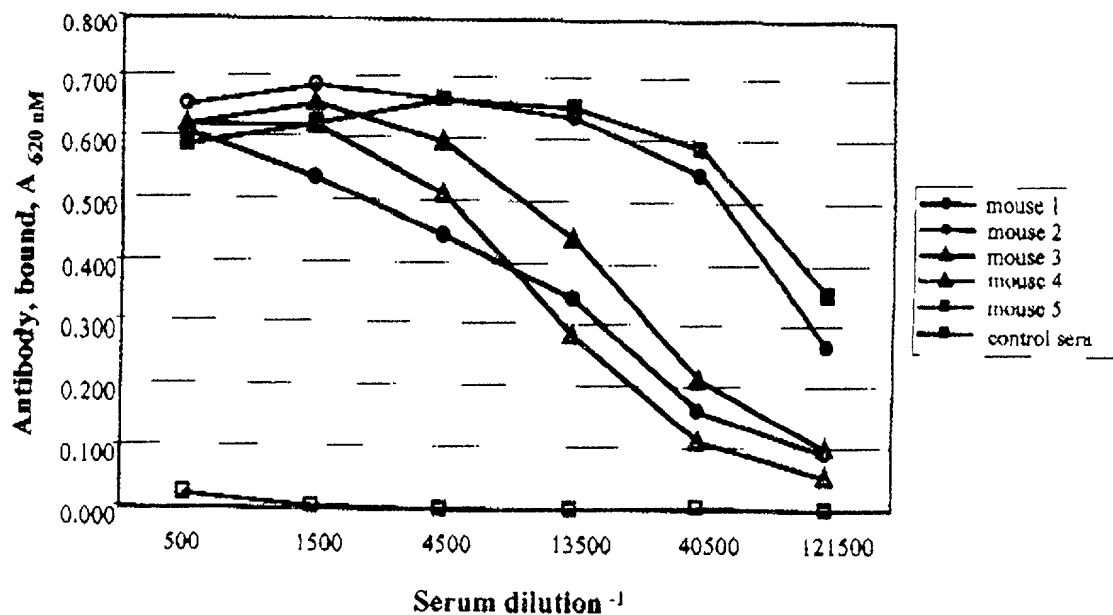


C.



**Biological activities of TSST-1 mutants.** a, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-TSST-1 antibody (affinity purified). b, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished activation of human lymphocytes; c, Introduction of an additional mutation, H135A to the TSST-1 mutant L30R results in the maximum reduction in T-cell stimulation. Human T-cell proliferation, was assessed by [3H]thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

FIGURE 8



**Antibody response to TSST-1 mutant L30R.** Mice received a total of three injections of vaccine (20 µg/mouse) in Alhydrogel, two weeks between injections. Sera were sampled two weeks after last vaccination and anti-TSST-1 specific antibody was measured by ELISA, using plates coated with wild-type TSST-1. Pooled non-immune mouse sera were used as negative control.

FIGURE 9

A.

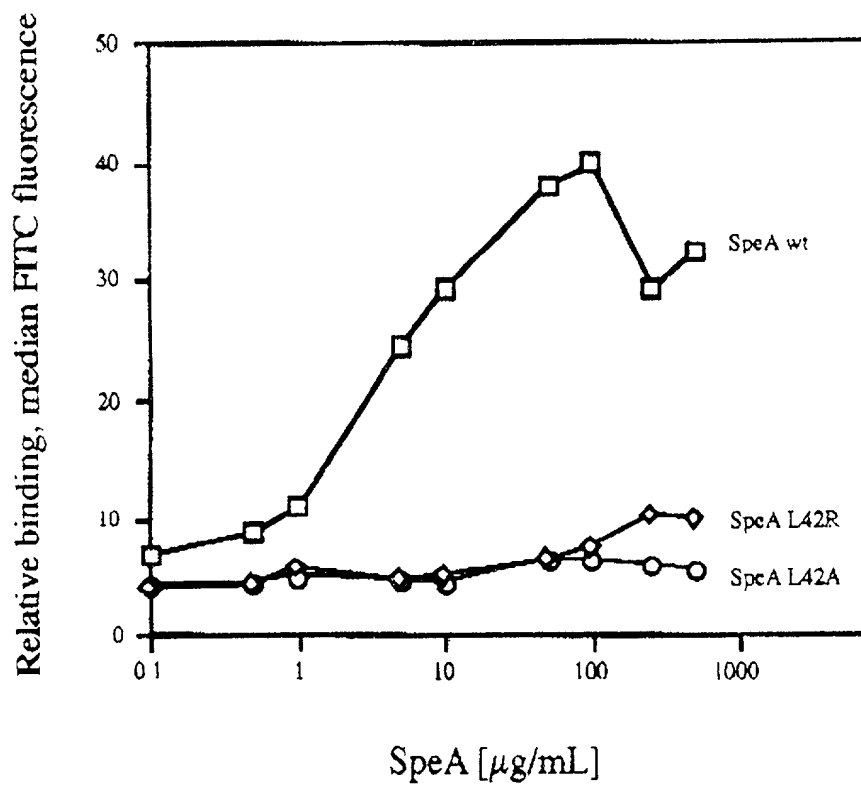
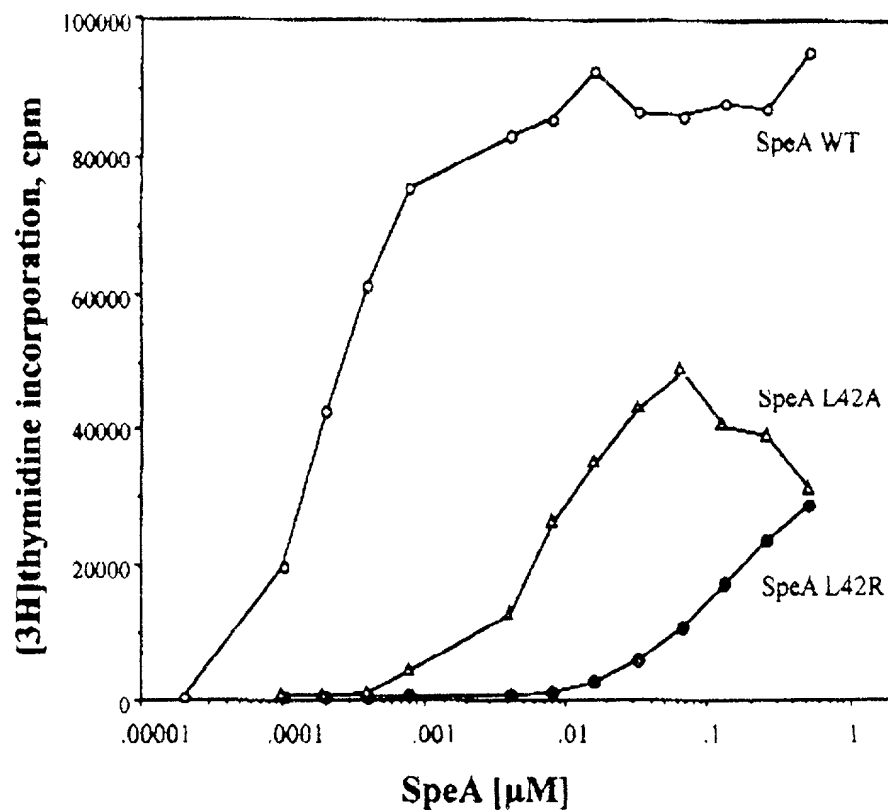


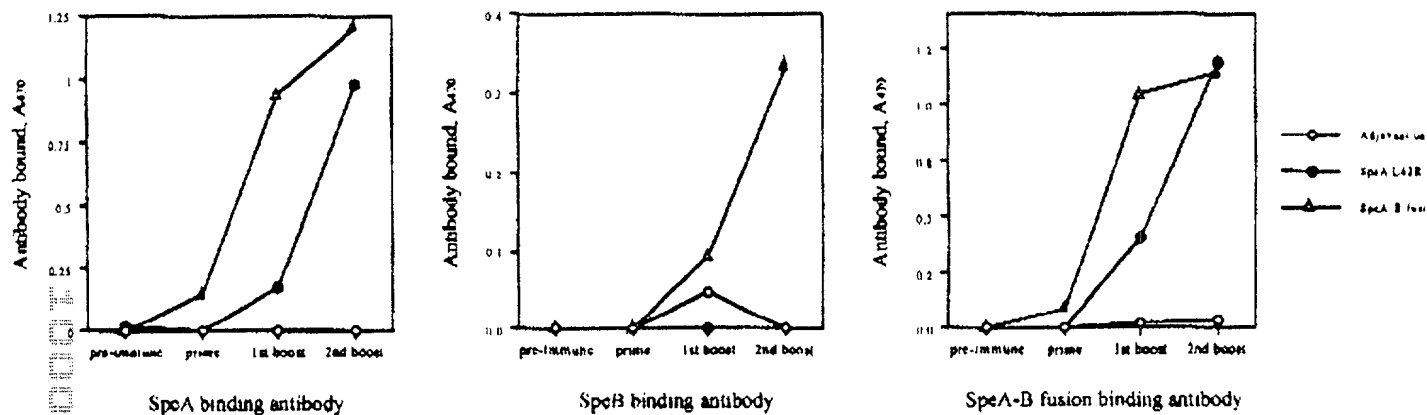
FIGURE 10

B.



**Biological activities of SpeA mutants.** a, Mutations of SpeA at amino acid position 42 (L42R) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-SpeA antibody (affinity purified). b Mutations of SpeA at amino acid position 42 (L42R or L42A) results in greatly diminished activation of human lymphocytes. Human T-cell proliferation, was assessed by [3H]thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

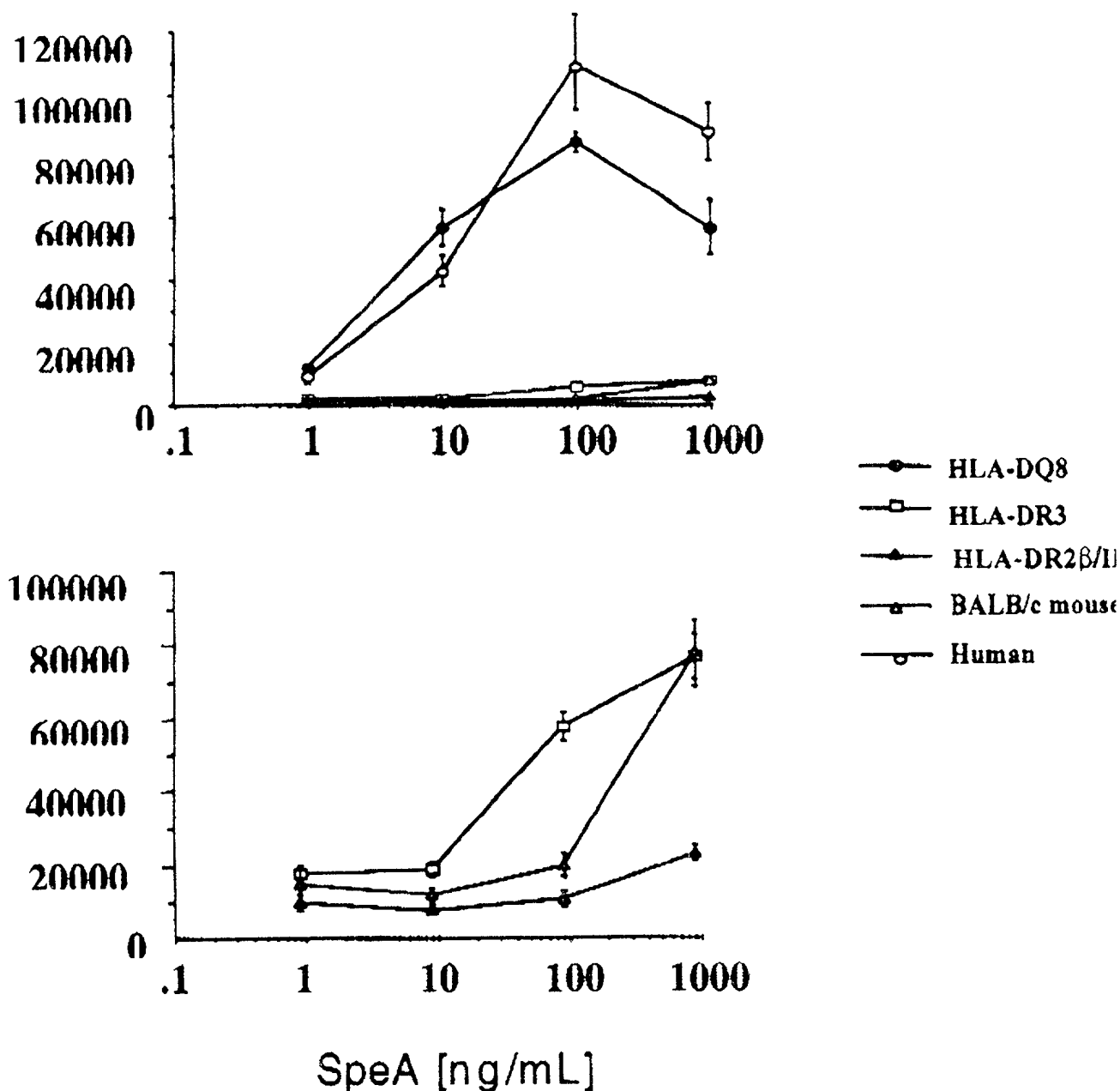
FIGURE 10



**Mouse antibody response to SpeA L42R and SpeA-B fusion constructs.** BALB/c mice were vaccinated three times with 10  $\mu$ g plus adjuvant (MPL<sup>TM</sup> + TDM+ CWS Emulsion, RIBI ImmunoChem Research, Inc., Hamilton, MT) of each construct, allowing two weeks between injections. Sera from each experimental group (n=5) were pooled for measurement of specific antibodies. Data shown are antigen-specific antibodies (ELISA units) present in a 1:100,000 dilution of pooled sera from mice vaccinated with SpeA L42R, SpeA-B fusion or adjuvant only.

FIGURE 11

[3H]thymidine incorporation, cpm



**T-cell response *in vitro* of mononuclear cells from transgenic mice expressing HLA-DQ8αβ and human CD4 closely approximate the physiological response of humans.** Mononuclear cells were isolated from spleens of transgenic mice expressing HLA-DR3, HLA-DQ8 or HLA-DR2β/1Eα, or non-transgenic BALB/c mice and human peripheral blood ( $4 \times 10^5$ /well). Following 60 h culture with SpeA, cells were pulse-labeled (12 h) with 1  $\mu$ Ci of [3H]thymidine. DNA from cells was harvested onto fiberglass filters and incorporated radioactivity measured by liquid scintillation.

FIGURE 12